ORIGINAL ARTICLE

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Expression and Survival Analysis Show High Mobility Group (HMG) Family as Prognostic Biomarkers in Breast Cancer

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ABSTRACT

Objective: High-mobility group (HMG) protein families are critical regulators of chromatin structure and gene expression in breast cancer. This study systematically evaluates their expression patterns, genetic interactions, and clinical relevance.

Materials and Methods: Expression profiles of HMG proteins were analyzed using mRNA data from gene expression profiling interactive analysis 2. We performed protein-level validation using the Human Protein Atlas. Prognostic significance was assessed through survival analysis, while genetic alterations were mapped using cBioPortal. Pathway enrichment and protein-protein interactions were explored with EnrichR and Search Tool for the retrieval of interacting genes/proteins, respectively. Associations with p53 mutation status were investigated using University of Alabama at Birmingham Cancer Data Analysis Portal.

Results: HMGA1 emerged as a central driver in triple-negative breast cancer (TNBC), forming a transcriptional complex with FOXM1 that activated VEGFA-mediated angiogenesis, which correlated withwas associated with poor patient survival. In contrast, HMGA2 overexpression was paradoxically associated with favorable outcomes despite promoting tumor angiogenesis. HMGB1 regulation was linked to genomic instability and metastasis, yet it showed potential protective effects in survival analyses. HMGB2 independently predicts poor prognosis in large tumors, and HMGB3 correlates with aggressive progression. HMGB4, though expressed at low levels, is associated with improved survival in early-stage patients. HMGN1 and HMGN4 promoted tumor growth, while HMGN2 suppressed proliferation and induced apoptosis, highlighting its therapeutic potential.

Conclusion: HMG proteins exhibit context-dependent roles in breast cancer, with HMGA1, HMGB2-3, and HMGN1/4 driving tumors, while HMGA2, HMGB1, HMGB4, and HMGN2 show protective or paradoxical effects. These findings position HMG proteins as both biomarkers and therapeutic targets, particularly HMGA1 in TNBC angiogenesis and HMGN2 in the induction of apoptosis.

Keywords: Breast cancer; HMG family; survival analysis; prognostic markers; mRNA expression

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KEY POINTS

- HMGA1 drives angiogenesis via FOXM1/VEGFA, which is associated with poor prognosis.
- HMGA2 paradoxically promotes angiogenesis, yet correlates with improved survival.
- HMGB1 up genomic instability/metastasis but may protect survival.
- HMGB2 promote tumour size/stage and HMGB3 signal advanced disease.
- HMGN1/HMGN4 promote tumour growth and HMGN2 inhibits proliferation and induces apoptosis.

Introduction

Breast cancer remains one of the most prevalent cancers among women worldwide and is a leading cause of cancer-related mortality (1). The complexity of breast cancer, characterized by its heterogeneous nature and varying responses to treatment, necessitates the identification of reliable prognostic markers (2). Early detection and accurate prognostic assessments are vital for improving survival rates and tailoring therapeutic strategies (3). Research has demonstrated that specific high mobility group (HMG) proteins are differentially expressed in breast cancer tissues compared with normal breast tissue, suggesting their involvement in tumorigenesis and progression (4).

HMG proteins are a diverse family of chromatin-associated proteins that play crucial roles in regulating gene expression, DNA repair, and cell proliferation (5). This family includes several members, each exhibiting distinct functions that contribute to cellular processes and tumor biology. HMGA (HMGA1, HMGA2) proteins show differentiation of embryonic and adult stem cells (6). HMGB proteins (HMGB1, HMGB2, HMGB3, HMGB4), first studied in yeast, have become central to cancer biology research. They regulate DNA repair and gene expression and drive cellular processes that promote tumour progression (7). HMG nucleosome-binding (HMGN) proteins, particularly HMGN1 and HMGN2, regulate chromatin accessibility and transcription factor binding in ameloblast differentiation. Their down-regulation accelerates ameloblast maturation and enamel deposition, highlighting their role in modulating gene expression, which may also influence cancer progression (8). HMGN3 promotes cancer progression by repressing epithelial regulatory genes in cholangiocarcinoma (CCA), with its high expression associated with metastasis (9). HMGN4 promotes thyroid cancer by altering gene expression, downregulating tumor suppressors, and increasing tumorigenicity (10).

In the context of breast cancer, these proteins have garnered attention due to their potential as prognostic biomarkers that can inform treatment decisions and predict patient outcomes.

The prognostic significance of HMG proteins in breast cancer is underscored by their associations with various clinical outcomes (11). Elevated levels of certain HMGA2 proteins have been associated with poor prognosis, whereas elevated levels of other HMGA2 proteins correlate with favourable outcomes (12).

Molecular cancer biomarkers, including those from the HMG protein family, serve as measurable indicators of cancer risk, presence, and patient prognosis (13). Their utility extends to risk assessment, screening, diagnosis, and monitoring of treatment responses. As the landscape of breast cancer treatment evolves, the integration of molecular biomarkers into clinical practice becomes increasingly important for personalized patient care (14).

Despite increasing recognition of the involvement of HMG proteins in oncogenesis, their specific contributions to breast cancer remain insufficiently defined. Existing omics-based studies have largely focused on individual gene signatures or pathway-level analyses, leaving the expression dynamics and prognostic relevance of HMG family members underexplored. Given their established roles in chromatin remodelling, transcriptional regulation, and tumour progression, HMG proteins represent promising yet under-investigated candidates for systematic evaluation in breast cancer. This study addresses this gap by integrating multi-omics datasets to comprehensively characterize HMG protein expression patterns across breast cancer subtypes and to assess their prognostic implications. By highlighting subtype-specific associations and utilizing large-scale online databases, we aim to identify novel biomarkers that could refine prognostic stratification. These biomarkers may also inform therapeutic strategies, thereby advancing precision oncology in breast cancer.

Materials and Methods

Gene Expression Patterns and Prognostic Evaluation of the HMG Family

We evaluated the mRNA expression levels of HMG proteins in breast cancer patients. This was performed using the gene expression profiling interactive analysis 2 (GEPIA) platform (http://gepia.cancer-pku.cn/) (15). Additionally, we performed a differential expression analysis based on pathological stages, considering a *p*-value of less than 0.01 significant. To confirm the correlation between mRNA and protein levels, we analyzed

HMG protein levels using the Human Protein Atlas database (https://www.proteinatlas.org/). We assessed the prognostic importance of HMG proteins using the Kaplan-Meier Plotter, which integrates survival data from Gene Expression Omnibus, European Genome-Phenome Archive, and the Cancer Genome Atlas (TCGA) for 943 breast cancer patients (16). To analyze overall survival (OS), progression-free survival (PFS), and post-progression survival (PPS), we categorized samples into high- and low-expression groups based on median expression levels. A univariate Cox regression analysis was conducted, adjusting for pathological grade, clinical stage, and TP53 mutation status. A *p*-value <0.05 was considered statistically significant.

Genetic Alteration Frequencies of HMG Family Proteins

We used the cBioPortal (http://www.cbioportal.org) for in breast cancer patients. This included selecting genomic profiles of HMG family members, focusing on mutations, copy-number alterations, and mRNA expression. The data obtained were used for further analysis and research.

Functional Enrichment Analysis of the HMG Family

We conducted a cancer hallmarks enrichment analysis of *HMG* genes linked to OS in human solid tumors. Additionally, we performed biological enrichment analysis, specifically for gene ontology (GO) terms and Reactome 2022 pathways, on HMG family members using EnrichR (17-20). These approaches helped us understand the role of *HMG* genes in biological processes.

Protein-Protein Interactions Analysis of HMG Family Members

To explore potential interactions among our genes of interest, we used the search tool for the retrieval of interacting genes (STRING) database (21). This tool identifies known and predicted protein-protein interactions from various sources, including experimental data and text mining. The gene list was input into STRING, and a confidence score threshold of 0.4 was applied to retain high-confidence interactions.

Gene Expression Analysis in Relation to p53 Mutation Status

We analyzed gene expression levels in relation to p53 mutation status using the University of Alabama at Birmingham Cancer Data Analysis Portal web tool (22, 23). This tool accesses gene expression data from TCGA database, allowing stratification by clinical and molecular characteristics, including tumor stage and p53 mutation status. We examined the data for statistically significant differences in expression levels between wild-type and mutant p53 samples. Gene expression levels were reported as transcripts per million (TPM) and visualized with box plots.

Ethical Statement

The data in this paper were sourced solely from an open-access database. We did not collect data directly from patients or interfere with their treatment. Therefore, ethical approval was not required for this study.

Results

HMG Proteins mRNA Expression Levels in Breast Cancer Patients

We examined the mRNA levels of HMG proteins in breast cancer patients. Our analysis using GEPIA revealed that the mRNA levels of HMGA1, HMGB1, HMGB2, HMGB3, HMGN1, HMGN2, HMGN3, and HMGN4 were significantly higher in breast cancer tissues compared with normal breast tissues (Figure 1). In contrast, HMGA2 exhibited moderate expression, while HMGB4 showed very low expression in cancer tissues (Figure 1). Furthermore, we investigated the expression of HMG proteins across different stages of breast cancer. We found that the mRNA levels of HMGA1, HMGB1, HMGB2, HMGB3, HMGN1, HMGN2, HMGN3, and HMGN4 differed significantly across tumor stages. However, the expression levels of HMGA2 and HMGB4 did not differ significantly across these stages (Figure 2). This suggests that certain HMG proteins may play distinct roles at different stages of cancer progression.

Prognostic Significance of HMG Proteins mRNA Expression Levels

The prognostic value of HMG proteins' mRNA levels in breast cancer patients was assessed using the Kaplan-Meier plotter. The results indicated that high mRNA levels of HMGA2, HMGB1, HMGB2, HMGB4, HMGN2, and HMGN4 were associated with better OS. Conversely, high levels of HMGA1, HMGB3, HMGN1, and HMGN3 correlated with poorer OS (Figure 3). The statistical analysis revealed significant differences among the HMGA, HMGB, and HMGN groups, highlighting the prognostic importance of these proteins. Additionally, high expression levels of HMGB1, HMGB4, HMGA2, and HMGN3, or low expression levels of HMGA1, HMGB2, HMGB3, HMGN1, HMGN2, and HMGN4, were correlated with favorable PFS (Figure 4). Patients exhibiting high expression levels of HMGB1 and HMGN2 showed favorable PPS. In contrast, high levels of HMGA1, HMGA2, HMGB2, HMGB3, HMGB4, HMGN1, HMGN3, and HMGN4 were associated with unfavorable PPS (Figure 5). These findings underscore the potential of HMG proteins as prognostic biomarkers in breast cancer.

The cancer hallmark overrepresentation table provided valuable insights into the roles of specific genes in various cancer hallmarks (Table 1). Among the hallmarks analyzed, sustaining proliferative signaling showed a notable association with HMGB1; however, the high *p*-value of 0.93429 indicates that its contribution to this hallmark may not be statistically significant. In contrast, genome instability presented a more compelling association: HMGB1 involvement exhibited an odds ratio of 3.01, suggesting a potential role in promoting genomic

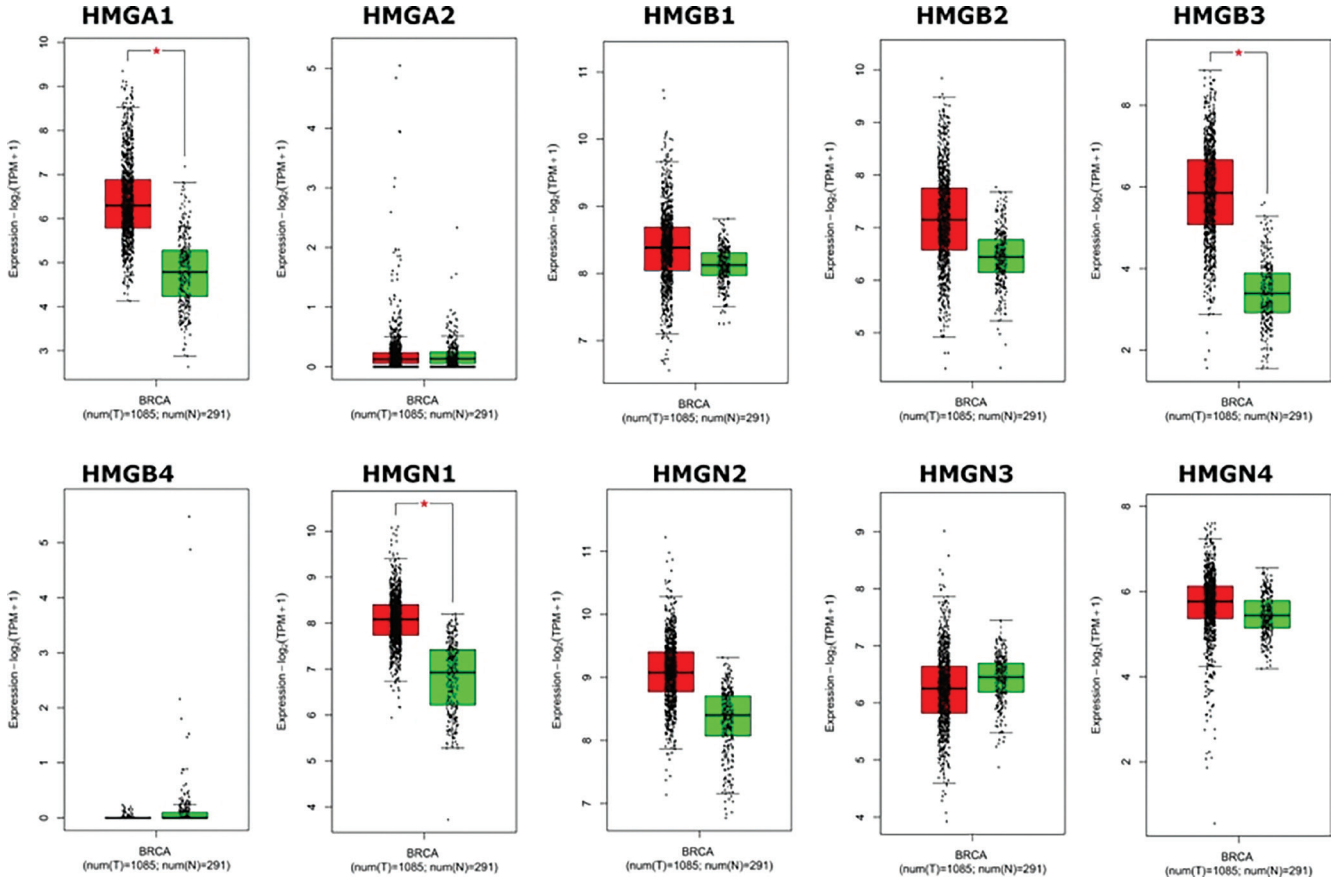

Figure 1. Differential mRNA expression patterns of HMG protein family members in BRCA tumor *vs*. normal tissue. Comparison of mRNA expression levels for HMG protein family members in breast cancer patients from TCGA dataset (n = 1085 tumors *vs*. 291 normal samples). Asterisks (*) indicate statistically significant differences (p<0.05) in expression between tumor (T) and normal (N) tissues. Proteins HMGA1/2, HMGB1–4, and HMGN1–4 demonstrate varied expression patterns across different HMG subgroups

HMG: High-mobility group; BRCA: Breast cancer; TCGA: The Cancer Genome Atlas; HMGN: HMG nucleosome-binding; TPM: Transcripts per million

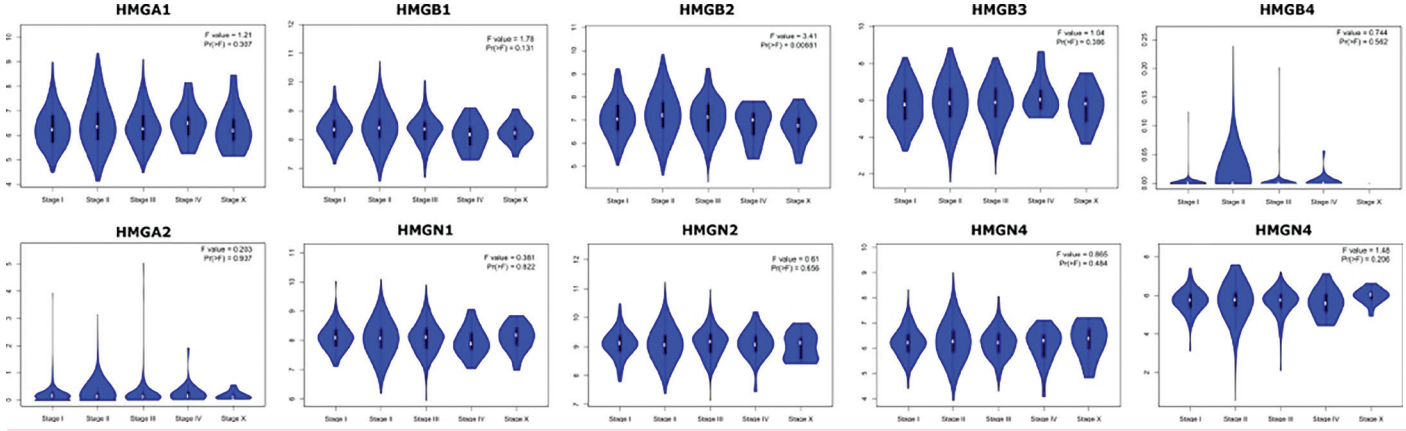

Figure 2. Stage-specific mRNA expression patterns of HMG proteins in breast cancer progression. Box plots were generated to compare HMG family mRNA expression across breast cancer progression stages (I-IV, X) in TCGA data, revealing stage-dependent variations in HMGA1/2, HMGB1–4, and HMGN1–4 subgroups. Notable findings include that HMGN4 shows significant stage-dependent expression (F = 3.41, p = 0.0088), whereas most members show non-significant trends (p>0.05)

HMG: High-mobility group; TCGA: The Cancer Genome Atlas; HMGN: HMG nucleosome-binding

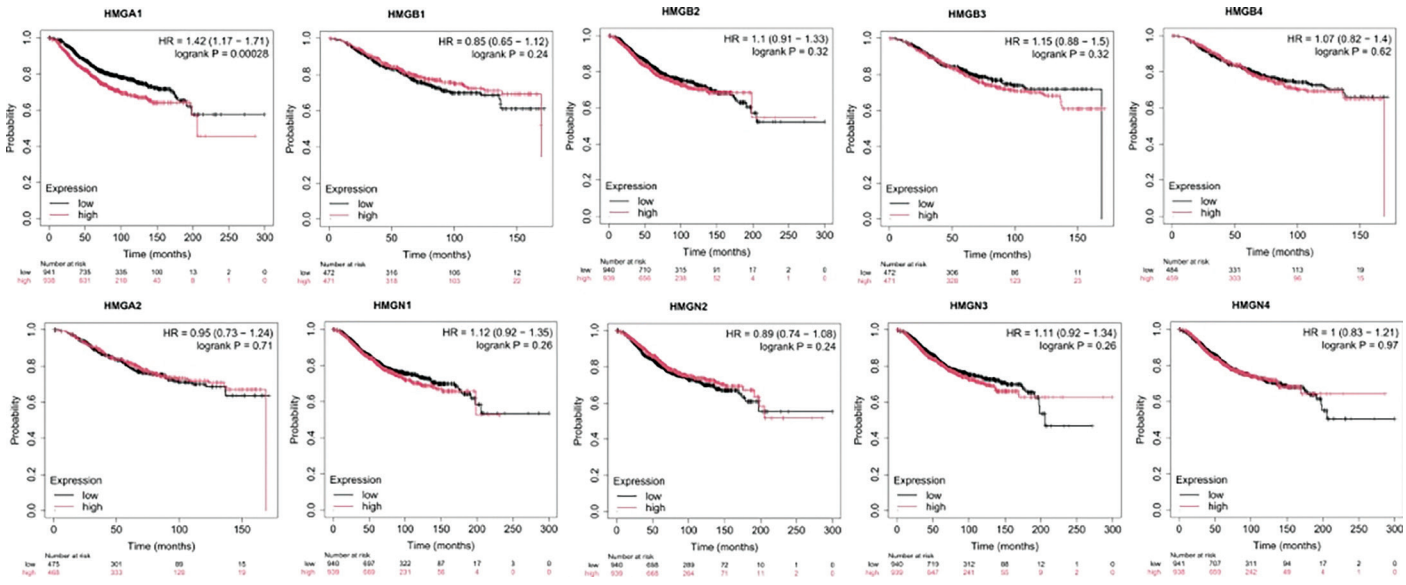

Figure 3. Survival analysis of HMG protein family mRNA expression in breast cancer patients. Kaplan-Meier survival analysis of HMG family mRNA [$\log_2$(TPM+1)] in the TCGA breast cancer cohort (n = 1,878) identifies HMGN1 as a key prognostic marker (HR = 1.42, p = 0.00028), with non-significant trends for HMGA4 (p = 0.62) and HMGB2 (p = 0.32). Log-rank test applied; group sizes shown

HMG: High-mobility group; TCGA: The Cancer Genome Atlas; HMGN: HMG nucleosome-binding; TPM: Transcripts per million; HR: Hazard ratio

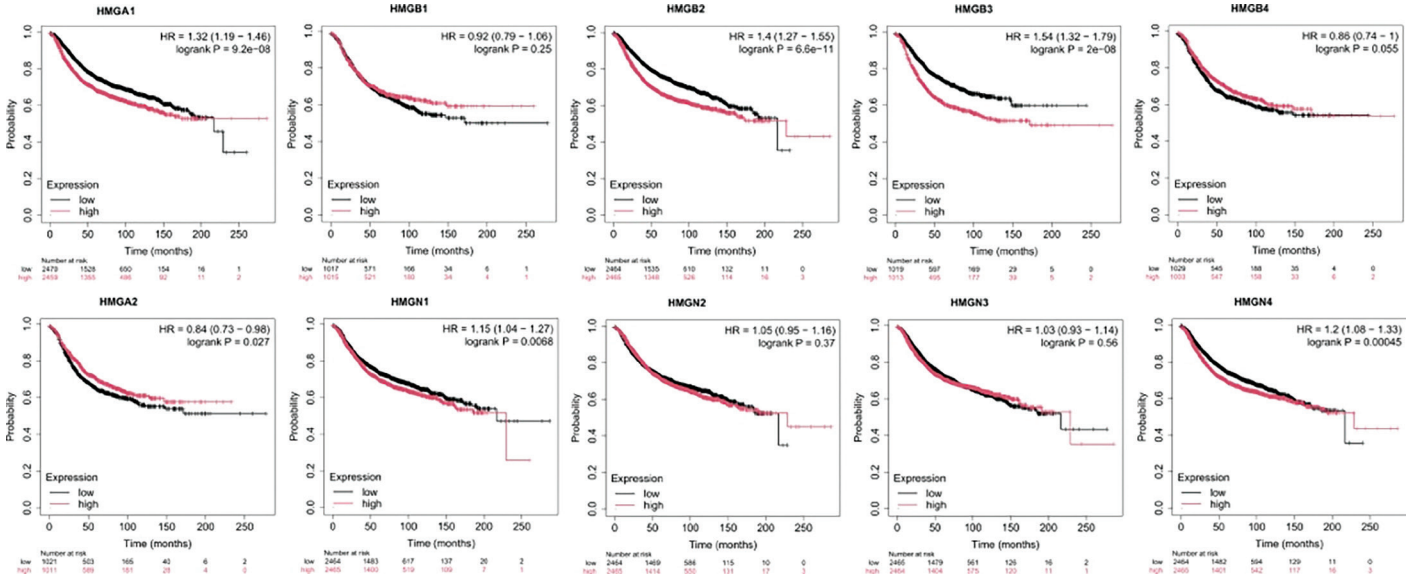

Figure 4. Prognostic impact of HMG family mRNA expression on breast cancer outcomes. Kaplan-Meier analysis of PFS among 2,465 TCGA breast cancer patients identifies divergent prognostic roles of HMG family members. HMGA1 and HMGN1 are associated with favorable outcomes, whereas HMGN4, HMGB4, and HMGN3 are associated with poorer prognosis; HMGN4 shows the strongest adverse prognostic effect. Non-significant trends are observed for HMGB2 and HMGA2

HMG: High-mobility group; TCGA: The Cancer Genome Atlas; HMGN: HMG nucleosome-binding; PFS: Progression-free survival

alterations that could drive cancer progression. The hallmark "evading growth suppressors" revealed notable interactions with HMGA1, HMGA2, and HMGB1, indicating that these genes may collectively contribute to bypassing regulatory mechanisms that typically inhibit cell proliferation. The odds ratio of 1.66 suggests a moderate association, reinforcing the idea that these genes could play a synergistic role in tumorigenesis. Sustained angiogenesis, critical for tumor growth, is associated with HMGA2, which has an odds ratio of 2.82, suggesting its significant involvement in promoting blood vessel formation to support tumor nourishment. HMGA2 and HMGB1 have been linked to the hallmark of tissue invasion and metastasis. Their involvement suggests a potential mechanism by which cancer cells invade surrounding tissues and spread to distant sites. The hallmarks of evading immune destruction, tumor-promoting inflammation, and resisting cell death did not significantly overlap with the analyzed genes. This absence of association suggests that these pathways may not be directly modulated by the genes under investigation

Prognostic Roles of HMG Proteins in Patients with Different Clinicopathological Features

This study examined the prognostic significance of HMG protein levels in breast cancer patients with varying clinicopathological features. We assessed the correlation of HMG protein expression with tumor stage and TP53 mutation status. We specifically investigated the expression levels of HMG proteins in relation to p53 mutation status, comparing wild-type and mutant p53 samples. Gene expression levels, quantified as TPM, were visualized using box plots, as presented in Figure 6. Notably, HMG proteins from all groups (HMGA, HMGB, and HMGN) exhibited elevated expression levels in p53-mutant samples, whereas the *HMGB4* gene was not expressed. This suggests that HMG proteins may have distinct roles based on p53 mutation status.

When stratified by cancer grade, the results showed (Table 2) that elevated levels of HMGB2, HMGB4, and HMGN1 mRNA were associated with better OS in patients with grade II disease. Conversely, high levels of HMGB4 and HMGN2 were linked to better OS in patients with grade I and grade III disease, while HMGB1 was associated with poor survival in grade I. High expression levels of all HMG proteins, except HMGB4, were associated with poor OS in stage I patients. This highlights the complexity of HMG protein roles in different cancer grades and stages.

HMG Family Genetic Alterations in Breast Cancer Patients

We evaluated the genetic alterations in the HMG family in breast cancer using cBioPortal. The frequency of gene alterations was

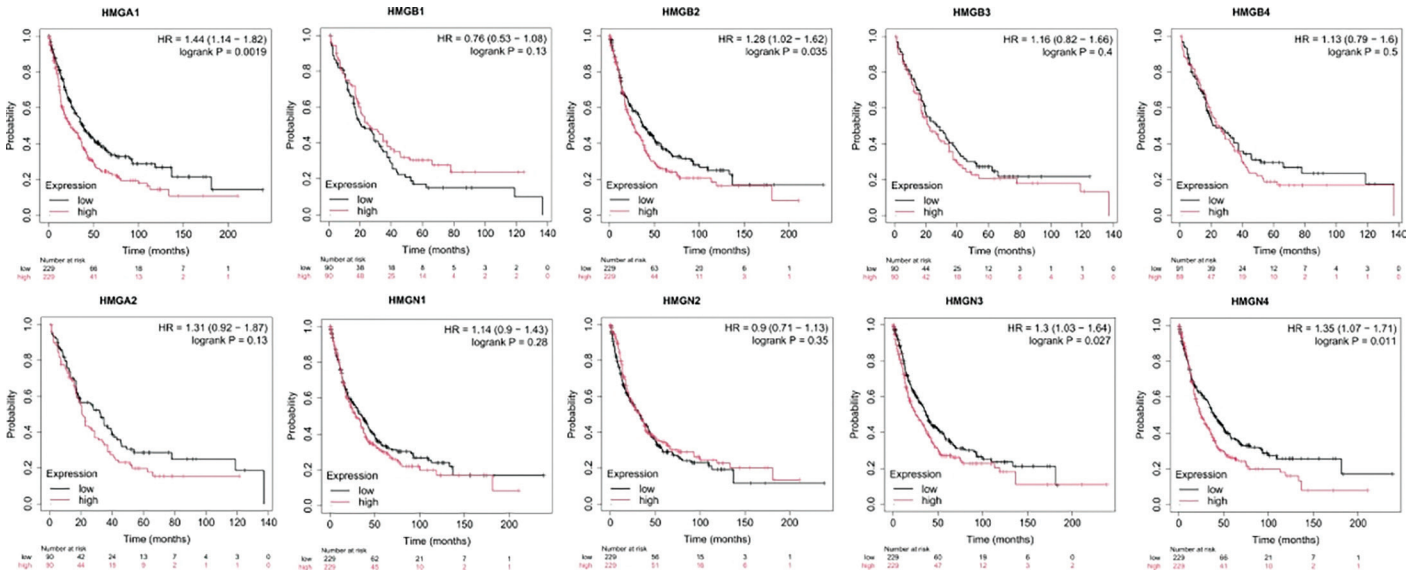

Figure 5. Post-progression survival analysis of HMG protein family mRNA expression in breast cancer. Kaplan-Meier curves showing post-progression survival outcomes stratified by mRNA expression levels of HMGA1/2, HMGB1–4, and HMGN1–4 in TCGA breast cancer cohort. Significant associations were observed for HMGN4 (HR = 1.35, 95% CI: 1.07–1.71, $p = 0.011$), HMGN2 (HR = 1.28, 95% CI: 1.02–1.62, $p = 0.035$), and HMGB1 (HR = 1.44, 95% CI: 1.14–1.82, $p = 0.0019$). Trends include HMGA4 (HR = 1.13, $p = 0.5$), and HMGB3 shows a statistically non-significant association (HR = 1.16, $p = 0.40$). The analysis was performed using the log-rank test with a median expression cut-off. Numbers at risk are shown for the low- and high-expression groups. Time scale: 0–200 months post-progression

HMG: High-mobility group; TCGA: The Cancer Genome Atlas; HMGN: HMG nucleosome-binding; HR: Hazard ratio; CI: Confidence interval

Table 1. HMG protein associations with cancer hallmark pathways

Cancer hallmark	Overlap	*p*-value	Adjusted *p*-value	Odds ratio	Hallmark *vs.* hallmark	Genes
Sustaining proliferative signaling	1/3574	0.93429	0.93429	0.5	0.39	*HMGB1*
Genome instability	1/747	0.4001	0.70848	3.01	1.88	*HMGB1*
Evading growth suppressors	3/3288	0.38067	0.70848	1.66	1.28	*HMGA2; HMGB1; HMGA1*
Evading immune destruction	0/1	Nan	1.0	Nan	0.0	Nan
Sustained angiogenesis	1/796	0.42041	0.70848	2.82	1.77	*HMGA2*
Tissue invasion and metastasis	2/2318	0.47232	0.70848	1.61	1.21	*HMGA2; HMGB1*
Tumor-promoting inflammation	0/1	Nan	1.0	Nan	0.0	Nan
Resisting cell death	1/1941	0.74997	0.89996	1.06	0.73	*HMGA2*
Reprogramming energy metabolism	0/1	Nan	1.0	Nan	0.0	Nan
Replicative immortality	0/1	Nan	1.0	Nan	0.0	Nan

Comprehensive enrichment analysis reveals heterogeneous involvement of HMG proteins across cancer hallmarks, though none reach statistical significance after multiple testing correction (FDR-adjusted $p>0.7$). The strongest raw associations were observed in evading growth suppressors (*HMGA1/HMGA2/HMGB1*; OR = 1.66) and tissue invasion and metastasis (OR = 1.61), while genome instability (OR = 3.01) and sustained angiogenesis (OR = 2.82) exhibited elevated risk trends. Five hallmarks, including immune evasion and metabolic reprogramming, showed no linkages to *HMG* genes

HMG: High-mobility group; FDR: False discovery rate; OR: Odds ratio

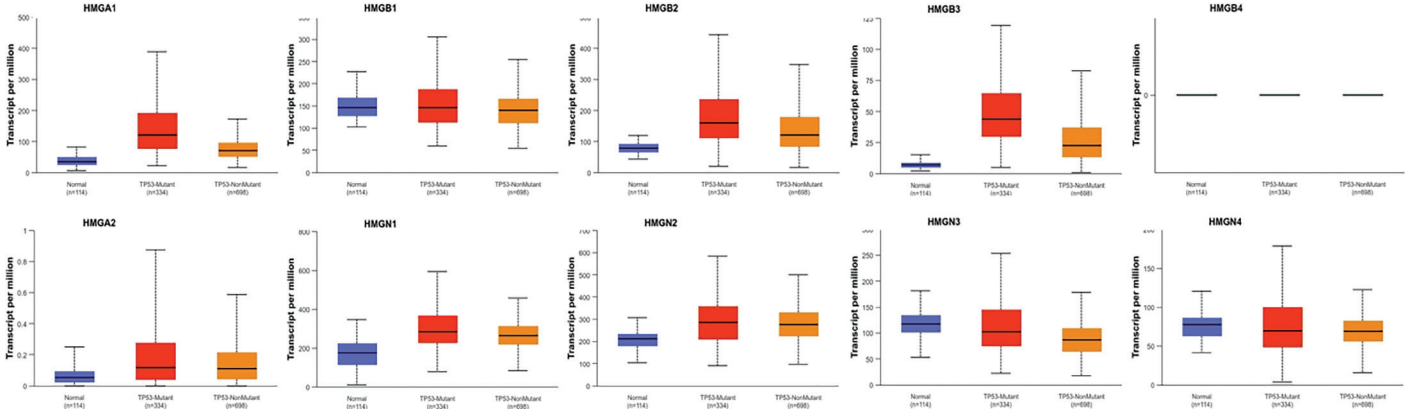

Figure 6. Differential expression patterns of HMG proteins by TP53 mutation status in breast cancer. Box plots comparing mRNA expression levels [$\log_2$ (TPM+1)] of HMGA1/2, HMGB1–4, and HMGN1–4 across normal breast tissue (n = 114), TP53-mutant (n = 334), and TP53-non-mutant (n = 698) tumors in TCGA cohort. HMGA1 (2.1-fold, mutant *vs.* non-mutant), HMGA2 (3.3-fold), and HMGB2 (1.8-fold) show upregulation in TP53-mutant tumors

HMG: High-mobility group; TCGA: The Cancer Genome Atlas; HMGN: HMG nucleosome-binding; TPM: Transcripts per million

measured as $\log_2$ values of the mutation count, ranging from 1 to 2. It includes mutations, amplifications, deep deletions, structural variants, and multiple alterations in TCGA datasets (Figure 7). *HMG* genes exhibited a high frequency of amplification, followed by mutations and deep deletions. Structural variants and multiple alterations occurred less frequently. The percentages of genetic alterations in specific *HMG* genes varied from <1.0% to 2.0% (e.g., HMGA1: 1.0%; HMGA2: 2.0%; HMGB1: 2.0%; HMGB2: 1.0%; HMGB3: 1.0%; HMGB4: <1.0%; HMGN1: 2.0%; HMGN2: <1.0%; HMGN3: <1.0%; HMGN4: 1.0%), with amplification being the most common alteration observed (Figure 8). We also assessed the prognostic roles of HMG in breast cancer patients with or without alterations. However, no significant correlation was found between the presence of alterations and OS or PFS in breast cancer patients (p-values: 0.140 and 0.112, respectively). Nonetheless, the unaltered group showed a favorable correlation with disease-free survival. The STRING database was used to construct a network of HMGs and closely interacting genes, demonstrating the intricate relationships between these proteins (Figure 8).

Table 2. Overall survival of breast cancer patients with different clinicopathological features and secretoglobin expression levels

Secretoglobin family	Clinicopathological features	Cases	HR	*p*-value
	Grades			
HMGA1	I	113	1.17	0.547
	II	243	1.07	0.529
	III	481	0.99	0.913
HMGA2	I	113	1.43	0.507
	II	243	1.25	0.371
	III	481	0.82	0.197
HMGB1	I	113	0.98	0.965
	II	243	0.80	0.380
	III	481	1.01	0.972
HMGB2	I	113	1.47	0.139
	II	243	1.27	**0.030**
	III	481	1.06	0.539
HMGB3	I	113	0.97	0.948
	II	243	1.23	0.417
	III	481	0.91	0.516
HMGB4	I	113	0.38	**0.093**
	II	243	0.43	**0.001**
	III	481	1.09	0.579
HMGN1	I	113	1.31	0.287
	II	243	1.21	**0.085**
	III	481	1.09	0.374
HMGN2	I	113	0.95	0.832
	II	243	1.03	0.776
	III	481	0.80	**0.016**
HMGN3	I	113	0.87	0.583
	II	243	1.08	0.479
	III	481	0.92	0.391
HMGN4	I	113	0.99	0.959
	II	243	0.94	0.559
	III	481	1.15	0.130

Survival analysis was used to evaluate the *p*-value. Bold font highlights the statistical significance of the difference; HR: Hazard ratio; HMG: High mobility group

Functional Enrichment Analysis of HMGs in Breast Cancer Patients

We utilized EnrichR to conduct enrichment analyses for GO Biological Process 2023, GO cellular component 2023, GO molecular function 2023, and Reactome 2022 on HMGs. The findings revealed that biological processes associated with HMGs were enriched in several categories, including protein-DNA complexes, chromatin organization, DNA conformation and topological changes, regulation of DNA binding, V(D)J recombination, regulation of stem cell proliferation, heterochromatin formation, and regulation of DNA-templated transcription (Table 3). The GO cellular component analysis indicated that HMGs primarily function in the nucleus, membrane-bounded intracellular organelles, and chromosomes, including heterochromatin and condensed nuclear chromosomes (Table 3). Molecular function enrichment analysis showed that HMGs are involved in DNA bending, formation of secondary structures and four-way junctions, and other critical processes.

The analysis found that the Reactome pathway associated with HMGs was enriched for several processes, including apoptosis induced DNA fragmentation, apoptotic execution phase, DNA damage/telomere stress induced senescence, APOBEC3G mediated resistance to human immunodeficiency virus type 1, two-long terminal repeat circle formation, programmed cell death, and integration of provirus (Table 3). These findings highlight the multifaceted roles of HMG proteins in breast cancer biology.

Discussion and Conclusion

HMGA1 is a master regulator in the progression of triple-negative breast cancer (TNBC), forming a complex with FOXM1 to enhance transcriptional activity of genes such as VEGFA, which promotes angiogenesis. Their co-expression correlates with poor patient prognosis, suggesting that targeting the HMGA1/FOXM1 interaction may be a viable therapeutic strategy (24). Our results showed significantly elevated HMGA1 mRNA levels in breast cancer tissues compared to normal tissues, indicating its potential role as an oncogene. Its association with poor OS in patients suggests that HMGA1 may contribute to tumor aggressiveness and progression. The involvement of HMGA1 in evading growth suppressors highlights its potential mechanism for bypassing regulatory pathways that typically inhibit cell proliferation. Given its prominent role in tumorigenesis, HMGA1 may serve as a critical biomarker for aggressive breast cancer phenotypes.

While HMGA2 exhibited moderate expression levels, its association with favorable survival outcomes indicates a complex role in breast cancer. The protein's involvement in sustained angiogenesis suggests it may facilitate tumor growth by promoting blood vessel formation. A study shows that HMGA2 is overexpressed in breast cancer, promoting cell proliferation, migration, and invasion while protecting against apoptosis. It also contributes to the acquisition of cancer stem cell features, suggesting that targeting HMGA2 may effectively impede breast cancer progression (25). This duality in function, acting as a promoter of angiogenesis while correlating with improved survival, necessitates further research to clarify its role in different tumor contexts and stages.

HMGB1 was significantly upregulated in breast cancer tissues and was strongly associated with genomic instability, suggesting a role

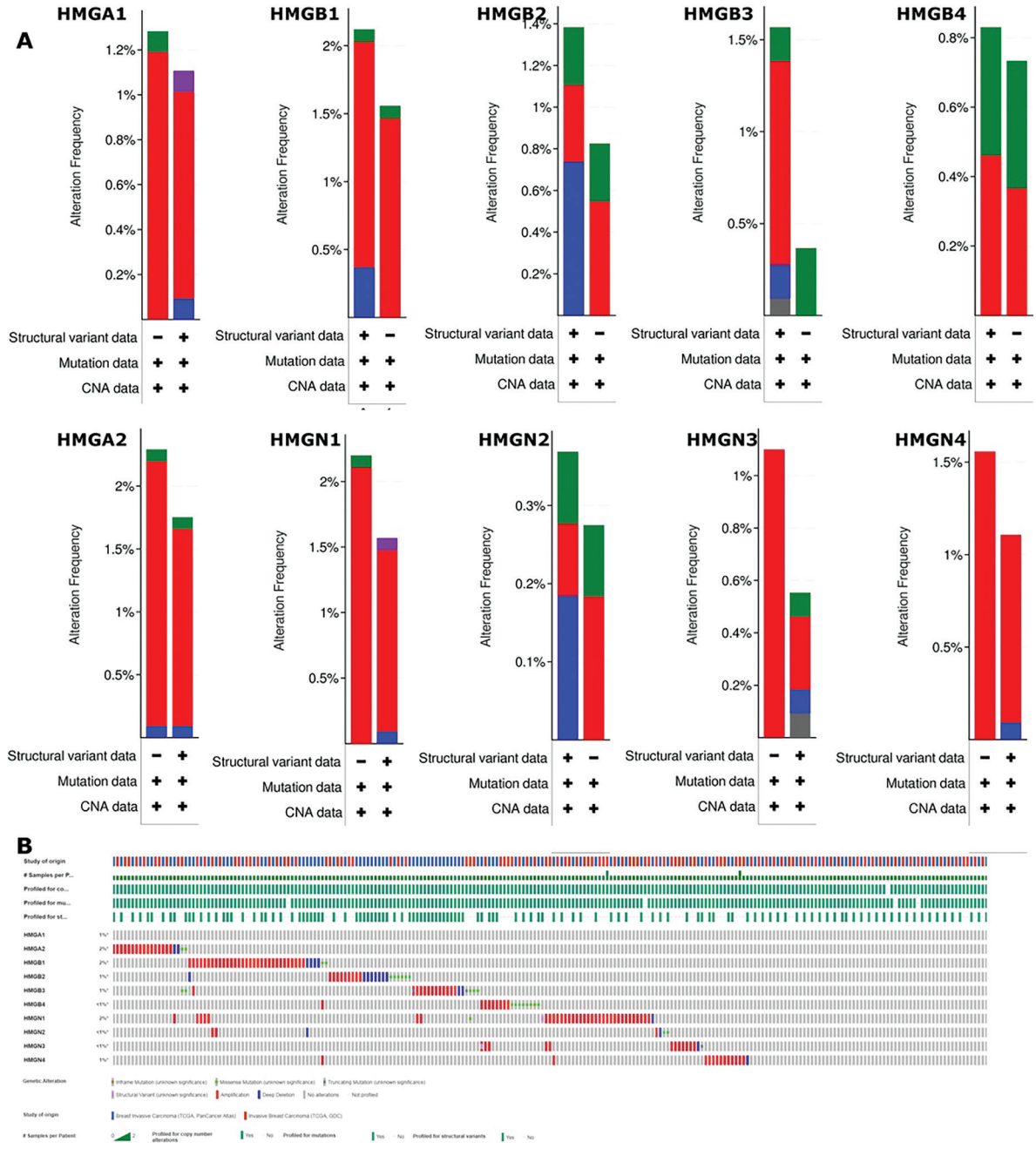

Figure 7. Genomic landscape of HMG protein family alterations in breast cancer. Analysis of 1,098 TCGA breast cancer patients reveals low yet patterns of genetic alterations in HMG-family genes. HMGA1 (2%) and HMGB2 (1.5%) show the highest alteration rates, and HMGA1 amplifications and HMGB3 deletions are the predominant copy-number changes. Structural variants in HMGN2/3 are rare (<0.5%), while HMGA2 mutations co-occur with TP53 alterations. Data integration from the TCGA PanCancer and GDC cohorts employs color-coded visualization (amplifications = red, deletions = blue) and platform-specific profiling indicators (+/– symbols)

HMG: High-mobility group; TCGA: The Cancer Genome Atlas; HMGN: HMG nucleosome-binding; GDC: Genomic data commons; CNA: Copy number alteration

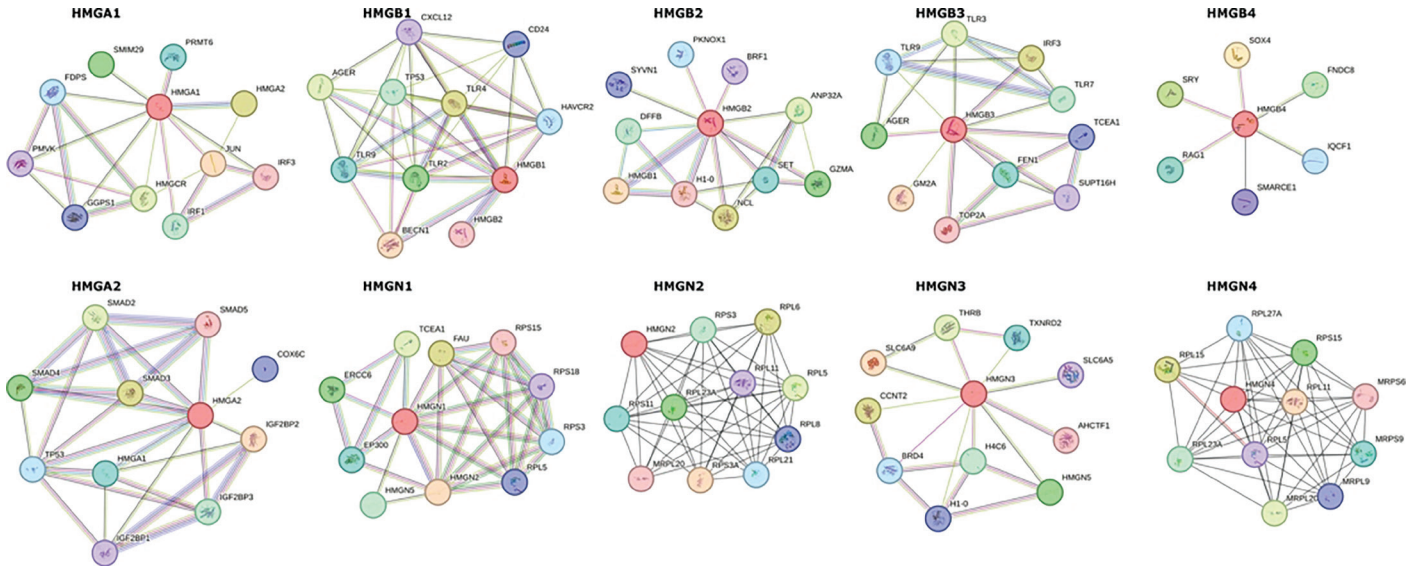

Figure 8. Protein interaction network of HMG family members in breast cancer biology. STRING analysis of HMG proteins reveals three core modules: (1) immune regulation via HMGB1/2-TLRs (2/3/4/9) and interferon genes; (2) chromatin remodeling through HMGA1/2-SMAD2–5/TP53 interactions; and (3) ribosome biogenesis linking HMGN1–4 to RPL/RPS proteins. Cross-cluster connections include HMGA1-DNA repair (*FEN1/ERCC6*) and HMGB4-cholesterol biosynthesis (*HMGCR/FDPS*)

HMG: High-mobility group; HMGN: HMG nucleosome-binding; STRING: Search tool for the retrieval of interacting genes/proteins; TLRs: Toll-like receptors; RPL/RPS: Ribosomal protein, large subunit/small subunit

Table 3. Gene ontology and pathways enrichment analysis of secretoglobin in breast cancer

Category	Term	*p*-value	*q*-value	Overlap genes
GO biological process 2023				
GOTERM_BP	Protein-DNA complex organization (GO: 0071824)	1.172228e-13	2.215510e-11	[*HMGN4, HMGB2, HMGA2, HMGN2, HMGN3, HMGN1*]
GOTERM_BP	Chromatin organization (GO: 0006325)	1.098989e-09	1.038544e-07	[*HMGN4, HMGB2, HMGA2, HMGN2, HMGN3, HMGN1*]
GOTERM_BP	DNA conformation change (GO: 0071103)	1.975326e-08	1.244455e-06	[*HMGB2, HMGB3, HMGB1*]
GOTERM_BP	DNA geometric change (GO: 0032392)	3.628342e-07	1.714392e-05	[*HMGB2, HMGB3, HMGB1*]
GOTERM_BP	Regulation of DNA binding (GO: 0051101)	1.538445e-06	5.815322e-05	[*HMGB2, HMGA2, HMGB1*]
GO cellular component 2023				
GOTERM_CC	Nucleus (GO: 0005634)	0.000011	0.000183	[*HMGN4, HMGA1, HMGB2, HMGA2, HMGB1, HMGN2, HMGN3, HMGB4, HMGN1*]
GOTERM_CC	Intracellular membrane-bounded organelle (GO: 0043231)	0.000040	0.000318	[*HMGN4, HMGA1, HMGB2, HMGA2, HMGB1, HMGN2, HMGN3, HMGB4, HMGN1*]
GOTERM_CC	Chromosome (GO: 0005694)	0.000062	0.000332	[*HMGB2, HMGA2, HMGB1*]
GOTERM_CC	Heterochromatin (GO: 0000792)	0.000261	0.001045	[*HMGA1, HMGA2*]
GOTERM_CC	Condensed chromosome (GO: 0000793)	0.000392	0.001255	[*HMGB2, HMGB1*]

Table 3. Continued

Category	Term	*p*-value	*q*-value	Overlap genes
GOTERM_CC	Nuclear chromosome (GO: 0000228)	0.046507	0.106379	[*HMGA2*]
GO molecular function 2023				
GOTERM_MF	DNA binding, bending (GO: 0008301)	2.918490e-17	1.109026e-15	[*HMGA1, HMGB2, HMGA2, HMGB3, HMGB1, HMGB4*]
GOTERM_MF	DNA secondary structure binding (GO: 0000217)	2.230118e-12	4.237225e-11	[*HMGA1, HMGB2, HMGA2, HMGB3, HMGB1*]
GOTERM_MF	Four-way junction DNA binding (GO: 0000400)	5.022899e-08	4.771754e-07	[*HMGB2, HMGB3, HMGB1*]
GOTERM_MF	Supercoiled DNA binding (GO: 0097100)	2.248131e-06	1.708580e-05	[*HMGB2, HMGB1*]
Reactome pathway 2022				
Reactome-2022	Apoptosis induced DNA fragmentation R-HSA-140342	0.000017	0.000869	[*HMGB2, HMGB1*]
Reactome-2022	Formation of senescence-associated heterochromatin foci R-HSA-2559584	0.000030	0.000869	[*HMGA1, HMGA2*]
Reactome-2022	Apoptotic execution phase R-HSA-75153	0.000294	0.005594	[*HMGB2, HMGB1*]
Reactome-2022	DNA damage/telomere stress induced senescence R-HSA-2559586	0.000405	0.005776	[*HMGA1, HMGA2*]
Reactome-2022	Cellular senescence R-HSA-2559583	0.002915	0.027539	[*HMGA1, HMGA2*]

GO: Gene ontology; GOTERM: Gene ontology term; BP: Biological process; CC: Cellular component; MF: Molecular function

in promoting genomic alterations that drive cancer progression. Notably, high levels of HMGB1 were linked to better overall and PFS, indicating its potential as a protective factor in breast cancer. HMGB1 enhances breast cancer metastasis by activating fibroblasts through the receptor for advanced glycation end products/aerobic glycolysis pathway. High HMGB1 expression in migratory cancer cells leads to increased fibroblast activation, which subsequently promotes tumor cell metastasis (26). Its involvement in evading growth suppressors further emphasizes its critical role in tumor biology, making it a promising candidate for therapeutic targeting.

HMGB2 is overexpressed in breast cancer tissues and correlates with larger tumor size and advanced stage. Its expression serves as an independent prognostic factor, promoting tumor growth and aerobic glycolysis by regulating lactate dehydrogenase B and fructose-1,6-bisphosphatase 1 (27). Findings show that the expression of HMGB2 is significantly higher in breast cancer tissues, and it is associated with favorable survival outcomes in certain tumor grades. Its role in chromatin organization and DNA binding suggests that HMGB2 may influence gene expression patterns critical for tumor development. The complex relationship between HMGB2 expression and patient survival underscores the need for further investigation into its functional mechanisms in breast cancer.

Similar to HMGB1 and HMGB2, HMGB3 was found to be upregulated in breast cancer tissues. However, its association with poor OS indicates that it may contribute to tumor progression. The role of HMGB3 in chromatin dynamics and gene regulation positions it as a key player in the molecular landscape of breast cancer. HMGB3 expression correlates positively with aggressive cancer features and negatively with hormone receptor status. Knockdown of HMGB3 promotes cancer cell proliferation and enhances sensitivity to chemotherapy (28). Understanding its specific contributions to tumor biology could reveal novel therapeutic strategies.

HMGB4 was expressed at very low levels in breast cancer tissues, yet its expression was associated with improved survival in certain patient subsets. This suggests that HMGB4 might have a protective role in early-stage disease. The lack of significant expression in advanced tumors indicates that it may not be a

primary driver of tumorigenesis but could still provide insights into tumor biology and patient prognosis.

HMGN1 expression was significantly elevated in breast cancer tissues and was associated with poorer OS. Its role in chromatin remodeling and transcriptional regulation suggests that HMGN1 may facilitate oncogenic processes. The association with unfavorable survival outcomes highlights the need for further exploration of its functional mechanisms and its potential as a therapeutic target. Previous studies have shown that HMGN1 regulates chromatin structure and tumour immunityto more than 147 proteins. These reported changes include effects on histone activity, stress pathways, and immune responses (29). The study shows HMGN1's critical role in cancer cell function and suggests potential avenues for therapeutic exploration based on proteomic analysis.

HMGN2 significantly inhibits proliferation and migration of Michigan Cancer Foundation-7 breast cancer cells and promotes apoptosis. *In vivo* studies using a mouse model demonstrated that administration of HMGN2 reduced tumor growth and increased apoptotic cell counts (30). Our findings show that increased expression of HMGN2 in breast cancer was associated with favorable survival outcomes in specific tumor grades. Its involvement in chromatin structure and transcriptional regulation positions it as a potential modulator of gene expression in cancer. The contrasting survival associations indicate that HMGN2 may play context-dependent roles in tumor biology.

HMGN3 was found to have elevated expression levels in breast cancer tissues, correlating with poor OS. Its role in chromatin dynamics suggests that it may influence gene expression patterns critical for tumor progression. The association with unfavorable survival outcomes emphasizes the need for further research to elucidate its precise role in breast cancer biology. HMGN3 has not been directly studied in breast cancer but has been highlighted for its role in CCA. It shows increased expression in CCA compared to hepatocellular carcinoma, suggesting both its potential as a diagnostic marker for CCA and its involvement in tumor invasion, which is influenced by transforming growth factor beta signaling (31).

HMGN4 is highly expressed in TNBC and regulates cell proliferation both *in vitro* and *in vivo*. It interacts with the signal transducer and activator of transcription 3 (STAT3) pathway, forming a feedback loop that promotes TNBC cell growth (32). Our study reported that HMGN4 exhibited increased expression in breast cancer tissues, but HMGN4 expression did not show a clear correlation with survival outcomes. Its involvement in chromatin organization and DNA binding indicates that it may regulate gene expression. Further studies are needed to clarify its functional significance in breast cancer and its potential as a prognostic marker.

This study highlights the diverse roles of HMG proteins (HMGA, HMGB, and HMGN) in breast cancer biology and prognosis. HMGA1 correlates with poor survival, underscoring its potential as a therapeutic target. HMGA2, despite being associated with favourable outcomes, promotes angiogenesis and tumour progression, reflecting its complex dual role. HMGB1 appears protective, as it is linked to improved survival, yet it simultaneously facilitates metastasis, whereas HMGB2 and HMGB3 are associated with aggressive disease features. HMGB4, expressed at low levels, may confer benefits in early-stage tumours. Among HMGN proteins, elevation of HMGN1 predicts poor prognosis, suggesting therapeutic relevance, whereas HMGN2 demonstrates anti-proliferative and pro-apoptotic effects. HMGN3 may contribute to progression, and HMGN4, highly expressed in TNBC, interacts with STAT3 signalling, though; its prognostic impact remains unclear. Collectively, these findings emphasize the translational potential of HMG proteins as biomarker panels for prognostic stratification and as therapeutic targets for pathway-specific interventions. Future validation through multi-cohort studies, functional assays, and clinical trials will be essential to establish their clinical utility and advance precision oncology in breast cancer.

Ethics

Ethics Committee Approval: Therefore, ethical approval was not required for this study.

Informed Consent: Informed consent from individuals was not required.

Footnotes

Authorship Contributions

Surgical and Medical Pracites: E.A.S., A.A., R.A.Z., A.A.W., E.B.A., R.E.A.S.; Concept: E.A.S., A.A.; Design: E.A.S., A.A., R.A.Z.; Data Collection and/or Processing: R.A.Z., A.A.W., E.B.A., R.E.A.S.; Analysis and/or Interpretation: E.A.S., A.A., R.A.Z., A.A.W., R.E.A.S.; Literature Search: E.A.S., A.A., R.A.Z., A.A.W., E.B.A., R.E.A.S.; Writing: E.A.S., A.A., R.A.Z., R.E.A.S.

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